

**ASSESSMENT OF BIOACTIVE CONSTITUENTS AND
ANTIDIABETIC ACTIVITIES OF *Nauclea latifolia* Sm. AND *Terminalia
catappa* L. LEAF EXTRACTS**

**IHEAGWAM, FRANKLYN NONSO
08CP07484**

SEPTEMBER, 2020

**ASSESSMENT OF BIOACTIVE CONSTITUENTS AND
ANTIDIABETIC ACTIVITIES OF *Nauclea latifolia* Sm. AND *Terminalia
catappa* L. LEAF EXTRACTS**

BY

IHEAGWAM, FRANKLYN NONSO

08CP07484

B.Sc, Biochemistry, Covenant University, Ota

M.Sc, Biochemistry, Covenant University, Ota

**A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE
STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF DOCTOR OF PHILOSOPHY, (Ph.D) IN
BIOCHEMISTRY IN THE DEPARTMENT OF BIOCHEMISTRY,
COLLEGE OF SCIENCE AND TECHNOLOGY, COVENANT
UNIVERSITY, OTA**

SEPTEMBER, 2020

ACCEPTANCE

This is to attest that this thesis is accepted in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy (Ph.D) in Biochemistry in the Department of Biochemistry, College of Science and Technology, Covenant University, Ota.

Mr. John A. Philip

(Secretary, School of Postgraduate Studies)

.....

Signature and Date

Prof. Abiodun H. Adebayo

(Dean, School of Postgraduate Studies)

.....

Signature and Date

DECLARATION

I, **IHEAGWAM, FRANKLYN NONSO (08CP07484)**, declare that this research was carried out by me under the supervision of Prof. Shalom N. Chinedu and Prof. Olubanke O. Ogunlana of the Department of Biochemistry, College of Science and Technology, Covenant University, Ota. I attest that this thesis has not been presented either wholly or partially for the award of any degree elsewhere. All the sources of materials and scholarly publications used in this thesis have been duly acknowledged.

IHEAGWAM, FRANKLYN NONSO

.....
Signature and Date

CERTIFICATION

We certify that this thesis titled “**Assessment of Bioactive Constituents and Antidiabetic Activities of *Nauclea latifolia* Sm. and *Terminalia catappa* L. Leaf Extracts**” is an original research work carried out by **IHEAGWAM, FRANKLYN NONSO (08CP07484)** in the Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria, under the supervision of Prof. Shalom N. Chinedu and Prof. Olubanke O. Ogunlana. We have examined and found the work acceptable as part of the requirements for the award of the degree of Doctor of Philosophy (Ph.D) in Biochemistry.

Prof. Shalom N. Chinedu
(Supervisor)

.....
Signature and Date

Prof. Olubanke O. Ogunlana
(Co-supervisor)

.....
Signature and Date

Prof. Olubanke O. Ogunlana
(Head of Department)

.....
Signature and Date

Prof. Ganiyu Oboh
(External Examiner)

.....
Signature and Date

Prof. Abiodun H. Adebayo
(Dean, School of Postgraduate Studies)

.....
Signature and Date

DEDICATION

I dedicate this work to God Almighty, the Author and finisher of my Faith, who, by His grace, has made possible the completion of this thesis. To Him, alone, be all the glory, power and adoration.

ACKNOWLEDGEMENTS

I will like to give my profound appreciation to God, the Author and finisher of my Faith, for the benefit and grace to run this Ph.D programme through to its completion. My immense gratitude also goes to the Chancellor of Covenant University, Dr. David O. Oyedepo, for his vision in establishing Hebron; a serene environment that aided the completion of this Ph.D programme. Many thanks to the Vice-Chancellor, Prof. Aderemi A. Atayero, the Deputy Vice-Chancellor, Prof. Akan B. Williams, the Registrar, Dr. Olusegun P. Omidiora, the Dean School of Postgraduate Studies, Prof. Abiodun H. Adebayo, the Sub-Dean School of Postgraduate Studies, Prof. Obinna C. Nwinyi, the Dean College of Science and Technology, Prof. Temidayo V. Omotosho and the entire management team of Covenant University for maintaining the Staff Development Scheme.

I am grateful to my Supervisor, Prof. Shalom N. Chinedu, for his encouragement, support, guidance, advice, and wisdom transferred throughout this Ph.D programme. I am forever appreciative of the time you made out for me despite your numerous official assignments. I won't forget your constant question in a hurry "what's happening?" and smiling at my answer "nothing much". I have learnt a lot from you not only as a mentor but as a father. The Lord will reward you accordingly in the fullness of his glory.

I also immensely thank Prof. Olubanke O. Ogunlana for the role she played as my Co-supervisor. Thank you for your untiring commitment to mentoring and encouraging me throughout the duration of this programme. Ma, you are more than a mother. Your eagle eye towards my manuscripts, as well as teaching me the nitty-gritty behind the rationale of writing will never be forgotten.

Deep appreciation goes to all the faculty, staff and students of Biochemistry and Biological Sciences Department, for their immense support, most especially to Dr. Solomon O. Rotimi,

who provided some reagents and started some discourse to motivate me in the course of this programme. To Opeyemi C. De Campos, Daniel U. Okere, Bose E. Adegboye and Mr. Alaba O. Adeyemi, I am grateful; your technical inputs into this research will not be forgotten. To the staff in the College Office, most especially my long-time friend, Samuel T. Popoola, I thank you all; your words of encouragement and support is highly appreciated. I deeply appreciate the family of Dr. Samuel A. Ejoh for helping out in numerous roles whenever needed. The Lord will continually make his to face shine upon your family.

Special thanks go to my parents Sir Charles K. Iheagwam and Lady Jovita I. Iheagwam, and my new found parents Mr. Emmanuel O. Onisile and Mrs. Toyin W. Niran-Onisile for their love, care, time, constant motivation and prayers towards the success of this programme. To my aunt and uncle, Mrs. Kate Iheagwam-Ahante and Ven. Andrew Iheagwam, you are most appreciated for your support and various input in the course of this programme. I also want to appreciate my brothers Nelson C. Iheagwam, Samuel A. Niran-Onisile and Solomon A. Niran-Onisile, who have helped to run a few errands to ensure there is no slack. To my lovely wife, prayer partner, manuscript editor and number one motivator, Mrs. Olawumi T. Iheagwam, and my son, Adriel A. Iheagwam, thank you for bearing with me all this while. Your sacrifice shall be rewarded in multiple folds. From my heart, “I love you all, and God bless you”.

I appreciate my bosom friends Joseph K. Odiba, Chijioke C. Onwuameze, Kenneth O. Joseph, Nonso O. Madueke, Ifeanyi A. Erem, Chisom Eboh and Olawale H. Ogunlana. Thank you for being steadfast whilst helping me do the needful. To the Raineri Ghost Football Club family, we have established a brotherhood with a bond to last a lifetime. Lastly, I appreciate Archbishop Vining Memorial Church Cathedral Youth Church (AVMCC), Daughters of Light AVMCC, Drama Ministry of AVMCC youth church, friends and well-wishers for their contributions. God Bless you all.

TABLE OF CONTENTS

	Page
COVER PAGE	i
TITLE PAGE.....	ii
ACCEPTANCE	iii
DECLARATION.....	iv
CERTIFICATION	v
DEDICATION.....	vi
ACKNOWLEDGEMENTS.....	vii
TABLE OF CONTENTS.....	ix
LIST OF FIGURES	xiv
LIST OF TABLES	xvi
LIST OF PLATES.....	xviii
LIST OF ABBREVIATIONS.....	xix
ABSTRACT	xxii
1 CHAPTER ONE: INTRODUCTION.....	1
1.1 Background to the Study.....	1
1.1.1 Study plants of interest	3
1.2 Statement of Research Problem.....	6
1.3 Research Questions.....	7
1.4 Aim and Objectives of the Study.....	7
1.5 Justification of the Study	8
2 CHAPTER TWO: LITERATURE REVIEW	10
2.1 Diabetes Mellitus	10
2.1.1 Risk factors	10
2.2 Type 1 Diabetes Mellitus.....	11
2.2.1 Epidemiology.....	11
2.2.2 Pathophysiology	14
2.2.3 Diagnosis and screening	15
2.2.4 Treatment and management.....	16
2.3 Type 2 Diabetes Mellitus.....	18
2.3.1 Epidemiology.....	18
2.3.2 Pathophysiology	18
2.3.3 Diagnosis and screening	19
2.3.4 Treatment and management.....	19
2.4 Gestational Diabetes Mellitus	20

2.4.1	Epidemiology.....	21
2.4.2	Pathophysiology	21
2.4.3	Diagnosis and screening	22
2.4.4	Treatment and management.....	23
2.5	Mechanistic Factors and Diabetes Mellitus Link	23
2.5.1	Oxidative stress.....	24
2.5.2	Inflammatory response	24
2.5.3	Insulin signalling pathways	25
2.6	Metabolic Changes Activated by Diabetes Mellitus.....	26
2.6.1	Polyol Pathway	27
2.6.2	Hexoseamine metabolism	28
2.6.3	Advanced glycation end products and dicarbonyl formation	28
2.6.4	Protein kinase C activation	29
2.6.5	Mammalian target of rapamycin-p70 S6 Kinase Pathway	29
2.7	Classification of DM Medications	30
2.7.1	Biguanide	30
2.7.2	Sulfonylureas	31
2.7.3	Thiazolidinedione (TZD).....	32
2.7.4	SGLT2 inhibitors	32
2.7.5	Incretin mimetics	33
2.8	Antidiabetics of Natural Sources	33
2.9	Antidiabetic Mechanisms of Characterised Compounds.....	36
2.9.1	α -glucosidase and α -amylase inhibition	36
2.9.2	Glucose transporters upregulation	38
2.9.3	Insulin secretagogues and proliferation	40
2.9.4	Oxidative stress amelioration	41
2.10	Computer-aided identification of antidiabetics from natural sources.....	42
3	CHAPTER THREE: MATERIALS AND METHODS.....	43
3.1	Materials	43
3.1.1	Chemicals and reagents	43
3.1.2	Collection and identification of plants.....	43
3.1.3	Experimental animals	44
3.1.4	Collection of blood samples	44
3.2	Methods	44
3.3	Preparation of Plant Extracts	44
3.3.1	Ethanol extraction.....	44
3.3.2	Aqueous extraction	44

3.4	Phytochemical Analyses	45
3.4.1	Qualitative estimation	45
3.4.2	Quantitative estimation	47
3.5	Identification of Phytoconstituents	49
3.5.1	Gas chromatography (GC) analyses	49
3.5.2	Mass spectroscopy (MS) analyses	50
3.6	<i>In vitro</i> Assessments	50
3.6.1	<i>In vitro</i> antioxidant assays	50
3.6.2	Human erythrocytes membrane stabilising assay	52
3.6.3	<i>In vitro</i> antidiabetic assay	53
3.7	<i>In vivo</i> Assessments	54
3.8	Experimental Designs	55
3.8.1	Acute toxicity assessment	55
3.8.2	Sub-Acute toxicity assessment	56
3.8.3	<i>In vivo</i> antidiabetic assessment	56
3.9	Experimental Procedures	59
3.9.1	Tissue collection	59
3.9.2	Tissue preparation	59
3.9.3	Analytical methods	60
3.9.4	Molecular biology assessments	74
3.9.5	Haematological analyses	74
3.9.6	Histopathological examination	75
3.10	<i>In silico</i> Analyses of Identified Compounds	75
3.10.1	Hardware and software	75
3.10.2	Ligand modelling	75
3.10.3	Protein preparation	76
3.10.4	Active site prediction	76
3.10.5	Virtual Screening	76
3.10.6	Molecular Docking	76
3.10.7	<i>In silico</i> analysis of drug-likeness	77
3.10.8	ADMET properties	77
3.11	Statistical Analyses	77
4	CHAPTER FOUR: RESULTS	78
4.1	Yield Quantitation	78
4.2	Phytochemical Analyses	78
4.2.1	Qualitative phytochemical analyses	78
4.2.2	Quantitative phytochemical analyses	78

4.2.3	Gas chromatography-mass spectroscopy (GC-MS) analyses	79
4.3	<i>In vitro</i> Antioxidant Assessment.....	88
4.3.1	DPPH radical scavenging ability	88
4.3.2	H ₂ O ₂ radical scavenging ability	88
4.3.3	Total antioxidant capacity (TAC)	88
4.3.4	Ferric reducing antioxidant power (FRAP)	88
4.4	<i>In vitro</i> Membrane Stabilising Assessments	89
4.4.1	Human erythrocytes membrane stabilising assay	89
4.5	<i>In vitro</i> Antidiabetic Assessments	95
4.5.1	α -amylase inhibitory activity	95
4.5.2	Mode of inhibition on α -amylase activity.....	95
4.5.3	α -glucosidase inhibitory activity	95
4.5.4	Mode of inhibition on α -glucosidase activity	96
4.6	Acute Toxicity Assessment	104
4.6.1	Effect of TCA single-dose treatment on animal and organ weight	104
4.6.2	Effect of TCA single-dose treatment on liver function	104
4.6.3	Effect of TCA single-dose treatment on kidney function.....	104
4.6.4	Effect of TCA single-dose treatment on lipid and insulin profile	104
4.6.5	Effect of TCA single-dose treatment on haematology	105
4.6.6	Effect of TCA single-dose treatment on organ pathology.....	105
4.7	Sub-Acute Toxicity Assessment	113
4.7.1	Effect of sub-acute 28-day TCA treatment on animal and organ weight ...	113
4.7.2	Effect of sub-acute 28-day TCA treatment on antioxidant activities	113
4.7.3	Effect of sub-acute 28-day TCA treatment on liver function	114
4.7.4	Effect of sub-acute 28-day TCA treatment on kidney function.....	114
4.7.5	Effect of sub-acute 28-day TCA treatment on other parameters	114
4.7.6	Effect of sub-acute 28-day TCA treatment on haematology	115
4.7.7	Effect of sub-acute 28-day TCA treatment on organ pathology.....	115
4.8	<i>In vivo</i> Antidiabetic Assessments	127
4.8.1	Effect of TCA treatment on animal and organ weight	127
4.8.2	Effect of TCA treatment on antioxidant activities.....	127
4.8.3	Effect of TCA treatment on liver function	128
4.8.4	Effect of TCA treatment on kidney function	129
4.8.5	Effect of TCA treatment on lipid profile	129
4.8.6	Effect of TCA treatment on other diabetes parameters	130
4.8.7	Effect of TCA treatment on insulin resistance and cardiovascular indexes	131
4.8.8	Effect of TCA treatment on haematology	131

4.8.9	Effect of TCA treatment on mRNA expression	132
4.8.10	Effect of TCA treatment on organ pathology	132
4.9	<i>In silico</i> Analyses of Identified Phytoconstituents	152
4.9.1	Ligand selection.....	152
4.9.2	Protein structure and active site identification.....	152
4.9.3	Virtual screening and molecular docking	155
4.9.4	Predicted drug-likeness.....	156
4.9.5	Predicted ADMET properties	156
5	CHAPTER FIVE: DISCUSSION	166
5.1	Yield of Leaf Extracts.....	166
5.2	Phytochemical Analyses	167
5.3	<i>In vitro</i> Antioxidant and Membrane Stabilising Analyses.....	169
5.4	<i>In vitro</i> Antidiabetic Analyses	172
5.5	<i>In vivo</i> Toxicity Analyses	175
5.6	<i>In vivo</i> Antidiabetic Analyses	178
5.7	<i>In silico</i> Analyses of Identified Phytoconstituents	187
6	CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS	191
6.1	Summary of Findings.....	191
6.2	Conclusion	193
6.3	Contributions to Knowledge	193
6.4	Limitations of the Study	194
6.5	Recommendations.....	194
	REFERENCES.....	195
	APPENDICES	222

LIST OF FIGURES

Figure	Title of Figures	Page
2.1	The natural history of T1DM.	12
2.2	The incidence of T1DM in children.	13
2.3	Pathogenesis of T1DM.	15
2.4	Progression of T1DM.	17
2.5	Pathogenic factors underlying GDM.	22
2.6	Hyperglycaemia- and hyperinsulinemia-induced metabolic pathway activation	27
2.7	Proposed molecular mechanisms of the blood-glucose lowering action of metformin.	31
2.8	Chemical structures of galegine and metformin.	34
2.9	Chemical structures of some SGLT inhibitors.	35
2.10	Chemical structures of fukugetin, GB2a, and GB2a glucoside.	37
2.11	Chemical structures of acarbose, miglitol, salacinol, kotalanol, and de-O-sulfonated kotalanol.	37
2.12	Chemical structures of active principles with GLUT-4 upregulation ability.	39
2.13	Quinovic acid glycosides and inulin-type fructans chemical structures.	40
2.14	Curcumin, cyanidin-3-O- β -D-glucopyranoside, berberine, apocynin and thymol chemical structures.	41
4.1	Inhibitory activity of <i>T. catappa</i> and <i>N. latifolia</i> leaf extracts on α -amylase activity.	97
4.2	Mechanism of inhibition on α -amylase activity by <i>N. latifolia</i> leaf extracts	98
4.3	Mechanism of inhibition on α -amylase activity by <i>T. catappa</i> leaf extracts	99
4.4	Inhibitory activity of <i>T. catappa</i> and <i>N. latifolia</i> leaf extracts on α -glucosidase activity.	100
4.5	Mechanism of inhibition on α -glucosidase activity by <i>N. latifolia</i> leaf extracts	101
4.6	Mechanism of inhibition on α -glucosidase activity by <i>T. catappa</i> leaf extracts	102
4.7	Effect of <i>T. catappa</i> aqueous extract on the total body weight gain in acute toxicological assessment.	106
4.8	Effect of <i>T. catappa</i> aqueous extract on relative organ weight in acute toxicological assessment.	107

4.9	Effect of <i>T. catappa</i> aqueous extract on total body weight gained in sub-acute toxicological assessment.	116
4.10	Effect of <i>T. catappa</i> aqueous extract on relative organ weight in sub-acute toxicological assessment.	117
4.11	Effect of <i>T. catappa</i> aqueous extract treatment on HFD/STZ-induced diabetic rats mean body weight changes during the experimental period.	133
4.12	Effect of <i>T. catappa</i> aqueous extract treatment on relative organ weight of HFD/STZ-induced diabetic rats.	134
4.13	Effect of <i>T. catappa</i> aqueous extract treatment on HFD/STZ-induced diabetic rats mean blood glucose changes during the experimental period.	141
4.14	Effect of <i>T. catappa</i> aqueous extract treatment on HFD/STZ-induced diabetic rat's oral glucose tolerance test on the last week of the experimental duration.	142
4.15	Effect of <i>T. catappa</i> aqueous extract treatment on cardiovascular indexes of HFD/STZ-induced diabetic rats.	143
4.16	Effect of <i>T. catappa</i> aqueous extract treatment on some insulin resistance indexes of HFD/STZ-induced diabetic rats.	144
4.17	Effect of <i>T. catappa</i> aqueous extract treatment on quantitative insulin-sensitivity check index (QUICKI) of HFD/STZ-induced diabetic rats.	145
4.18	Effect of TCA treatment on β -cell index of HFD/STZ-induced diabetic rats.	146
4.19	Graphical representation of (a) GLUT-4 (b) DPP-IV (c) IRS1 d) Nrf2 (e) IL-6 and (f) TNF- α mRNA expression in the liver of experimental rats	149
4.20	3D structure and predicted binding sites of (a) α -amylase (b) α -glucosidase (c) DPP-IV	154

LIST OF TABLES

Table	Title of Tables	Page
3.1	Normal and high-fat diet chow formulation	58
3.2	Primer sequences used for reverse transcriptase-polymerase chain reaction	74
4.1	Qualitative phytochemical constituents and yield of <i>N. latifolia</i> and <i>T. catappa</i> extracts	81
4.2	Total flavonoid, phenolic, tannin, β -carotene, lycopene and alkaloid content of <i>N. latifolia</i> and <i>T. catappa</i> leaf extracts.	82
4.3	Biochemical compounds identified in <i>N. latifolia</i> ethanol leaf extract	83
4.4	Biochemical compounds identified in <i>N. latifolia</i> aqueous leaf extract	84
4.5	Biochemical compounds identified in <i>T. catappa</i> ethanol leaf extract	85
4.6	Biochemical compounds identified in <i>T. catappa</i> aqueous leaf extract	86
4.7	Classification of biochemical compounds identified from <i>N. latifolia</i> and <i>T. catappa</i> leaf extracts	87
4.8	DPPH radical scavenging ability of <i>N. latifolia</i> and <i>T. catappa</i> leaf extracts and standards	90
4.9	H ₂ O ₂ radical scavenging ability of <i>N. latifolia</i> and <i>T. catappa</i> leaf extracts and standards.	91
4.10	Total antioxidant capacity of <i>N. latifolia</i> and <i>T. catappa</i> leaf extracts.	92
4.11	Ferric reducing antioxidant power of <i>N. latifolia</i> and <i>T. catappa</i> leaf extracts.	93
4.12	Inhibitory effect of <i>N. latifolia</i> and <i>T. catappa</i> leaf extracts on hypotonic solution-induced haemolysis of erythrocyte membrane	94
4.13	IC ₅₀ , Vmax and Km values of <i>N. latifolia</i> and <i>T. catappa</i> leaf extracts on α -glucosidase and -amylase.	103
4.14	Effect of <i>T. catappa</i> aqueous extract single dose treatment on some liver function, kidney function and lipid profile parameters	108
4.15	Effect of <i>T. catappa</i> aqueous extract single dose treatment on haematological parameters	109
4.16	Effect of <i>T. catappa</i> aqueous extract on superoxide dismutase (SOD), peroxidase (Px) and glutathione-S-transferase activity in sub-acute toxicological assessment	118
4.17	Effect of <i>T. catappa</i> aqueous extract on reduced glutathione (GSH) and lipid peroxidation (MDA) level in sub-acute toxicological assessment	119

4.18	Effect of <i>T. catappa</i> aqueous extract on some liver function, kidney function and lipid profile parameters in sub-acute toxicological assessment	120
4.19	Effect of <i>T. catappa</i> aqueous extract on plasma and organ protein in sub-acute toxicological assessment	121
4.20	Effect of <i>T. catappa</i> aqueous extract on haematological parameters in sub-acute toxicological assessment	122
4.21	Effect of <i>T. catappa</i> aqueous extract treatment on superoxide dismutase (SOD), peroxidase (Px) and glutathione-S-transferase activities in HFD/STZ-induced diabetic rats	135
4.22	Effect of <i>T. catappa</i> aqueous extract treatment on reduced glutathione (GSH) and lipid peroxidation (MDA) concentrations in HFD/STZ-induced diabetic rats.	136
4.23	Effect of <i>T. catappa</i> aqueous extract treatment on liver and kidney function parameters in HFD/STZ-induced diabetic rats.	137
4.24	Effect of <i>T. catappa</i> aqueous extract treatment on lipid profile parameters in HFD/STZ-induced diabetic rats.	138
4.25	Effect of <i>T. catappa</i> aqueous extract treatment on other biochemical parameters in HFD/STZ-induced diabetic rats.	140
4.26	Effect of <i>T. catappa</i> aqueous extract treatment on haematological parameters in HFD/STZ-induced diabetic rats.	147
4.27	Selected GCMS identified phytoconstituents and their structures	153
4.28	Virtual screening results of identified ligands on α -amylase using iGEMDOCK	158
4.29	Virtual screening results of identified ligand on α -glucosidase using iGEMDOCK	159
4.30	Virtual screening results of identified ligand on DPP-IV using iGEMDOCK	160
4.31	Molecular docking results of virtually screened hits on α -amylase, α -glucosidase and DPP-IV using Autodock Vina	161
4.32	Physicochemical parameters of potential hit compounds identified from <i>N. latifolia</i> and <i>T. catappa</i> extracts and their comparison with Lipinski rule of drug-likeness.	163
4.33	Predicted pharmacokinetic and toxicity properties of potential lead compounds identified from <i>N. latifolia</i> and <i>T. catappa</i> extracts	164

LIST OF PLATES

Plate	Title of Plates	Page
1.1	Picture of <i>Nauclea latifolia</i> Sm.	5
1.2	Picture of <i>Terminalia catappa</i> L.	6
4.1	Histopathological examination of a) control b) 1000 mg/kg bwt c) 2500 mg/kg bwt d) 5000 mg/kg bwt hepatic tissues after <i>T. catappa</i> aqueous extract single dose administration.	110
4.2	Histopathological examination of a) control b) 1000 mg/kg bwt c) 2500 mg/kg bwt d) 5000 mg/kg bwt renal tissues after <i>T. catappa</i> aqueous extract single dose administration.	111
4.3	Histopathological examination of a) control b) 1000 mg/kg bwt c) 2500 mg/kg bwt d) 5000 mg/kg bwt spleen tissues after <i>T. catappa</i> aqueous extract single dose administration.	112
4.4	Histopathological examination of a) control b) 200 mg/kg bwt c) 400 mg/kg bwt d) 800 mg/kg bwt hepatic tissues after 28-day sub-acute <i>T. catappa</i> aqueous extract administration.	123
4.5	Histopathological examination of a) control b) 200 mg/kg bwt c) 400 mg/kg bwt d) 800 mg/kg bwt renal tissues after 28-day sub-acute <i>T. catappa</i> aqueous extract administration.	124
4.6	Histopathological examination of a) control b) 200 mg/kg bwt c) 400 mg/kg bwt d) 800 mg/kg bwt spleen tissues after 28-day sub-acute <i>T. catappa</i> aqueous extract administration.	125
4.7	Histopathological examination of a) control b) 200 mg/kg bwt c) 400 mg/kg bwt d) 800 mg/kg bwt cardiac tissues after 28-day sub-acute <i>T. catappa</i> aqueous extract administration.	126
4.8	Agarose gel photograph of (a) GLUT-4 (b) DPP-IV (c) IRS1 d) Nrf2 (e) IL-6 and (f) TNF- α mRNA expression in the liver of experimental rats.	150
4.9	Histopathological examination of a) control b) diabetic group (c) glibenclamide c) 400 mg/kg bwt treated and d) 800 mg/kg bwt treated hepatic tissues after 28-day <i>T. catappa</i> aqueous extract treatment of diabetic rats.	151

LIST OF ABBREVIATIONS

4-PL	Four-parameter logistic curve-fit
AAE	Ascorbic acid equivalent
AGE	Advanced glycation end product
AGEs	Advanced glycosylated end products
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AMP	Adenosine monophosphate
AMPK	Adenosine monophosphate-activated protein kinase
AMPK	Adenosine monophosphate-activated protein kinase
AST	Aspartate transaminase
BHT	Butylated hydroxytoluene
c-AMP	Cyclic Adenosine monophosphate
CDNB	Chloro-2,4-dinitrobenzene
CHOL	Total cholesterol
CHREC	Covenant University Health, Research and Ethics Committee
CISI	Composite insulin sensitivity index
CEL/CML	N-ε-carboxyethyl-lysine/N-ε-carboxymethyl-lysine
CPT	Carnitine palmitoyl transferase
CRI	Coronary risk Index
CVD	Cardiovascular disease
DCs	Dendritic cells
DM	Diabetes mellitus
DMSO	Dimethyl sulfoxide
DPPH	2,2-Diphenyl-2-picrylhydrazyl
DPP-IV	Dipeptidyl peptidase-4
DPP-IVi	DPP-IV inhibitors
ERK1/2	Extracellular signal-regulated kinase 1/2
EDTA	Ethylenediaminetetraacetic acid
FRAP	Ferric reducing antioxidant power
FRIN	Forest Research Institute of Nigeria
GA	Gallic acid

GAE	Gallic acid equivalent
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectroscopy
GDM	Gestational diabetes mellitus
GHS	Globally harmonised classification system
GIP	Glucose-dependent insulintropic polypeptide
GlcN-6-P	Glucosamine-6-phosphate
GLP-1	Glucagon-like peptide
GLUT	Glucose transporters
H ₂ O ₂	Hydrogen peroxide
HbA1c	Haemoglobin A1c
HDL	High-density lipoprotein
HOMA	Homeostasis model assessments
hs-CRP	High sensitive C-reactive protein
HTR	HDL-TRIG ratio
IDF	International Diabetes Foundation
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IKK-β	Inhibitor of nuclear factor kappa-B kinase beta
IR	Insulin resistance
IRs	Insulin receptors
IST	Insulin signal transduction
K _{ATP}	ATP-sensitive potassium channels
Keap-1	Kelch-like ECH-associated protein 1
LUTH	Lagos University Teaching Hospital
MG-H1	methylglyoxal-derived hydroimidazolone 1
MPs	Medicinal plants
MS	Mass spectroscopy
NIMR	National Institute of Medical Research
NIST	National Institute of Standards and Technology
NL	<i>Nauclea latifolia</i>
NLR	Neutrophil-to-lymphocyte ratio
NOAEL	No observed adverse effect level

NSAIDS	Non-steroidal anti-inflammatory drugs
OECD	Organization for Economic Cooperation and Development
OGCT	Oral glucose challenge test
OGTT	Oral glucose tolerance test
PCR	Polymerase chain reaction
PIGF	Placenta growth factor
pNPG	p-Nitrophenyl- α -D-glucopyranoside
PPAR γ	Peroxisome proliferative-activated receptor gamma
PIP2	Phosphatidylinositol 4,5-bisphosphate
PIP3	Phosphatidylinositol 3,4,5-trisphosphate
QUIKI	Quantitative insulin-sensitivity check index
RBC	Red blood cells
RE	Rutin equivalent
RO5	Lipinski rule of five
ROS	Reactive oxygen species
RTg	Renal threshold for glucose
RT-PCR	Reverse transcriptase-polymerase chain reaction
SGLT	Sodium-glucose cotransporter
SHBG	Sex hormone binding globulin
STZ	Streptozotocin
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
T3DM	Type 3 diabetes mellitus
TAC	Total antioxidant activity
TAE	Tannic acid equivalent
TC	<i>Terminalia catappa</i>
TFC	Total flavonoid content
TP	Total protein
TPC	Total phenolic content
TRIG	Triglycerides
TTC	Total tannin content
TZD	Thiazolidinedione
USFDA	United States Food and Drug Administration

ABSTRACT

Nauclea latifolia (NL) and *Terminalia catappa* (TC) leaves are used by locals in Nigeria to treat diabetes. However, there is paucity of scientific data on the antidiabetic activities and molecular mechanisms of action of these plants; hence, the set objectives of this research work. Samples of NL and TC leaves were collected from Ibadan in Oyo State and Ota in Ogun State, respectively, and identified. Aqueous (A) and ethanol (E) crude extracts of the plants were prepared for the analyses. Phytochemical analyses, *in silico* simulation, *in vitro* antidiabetic and membrane stabilising assessments were carried out using standard methods. Phytoconstituent assessment of NL and TC leaves using gas chromatography-mass spectroscopy (GC-MS) revealed the presence of 50 and 38 different phytochemicals, respectively. These were categorized as alcohols, alkaloids, carbohydrates, hydrocarbons, carboxylic acids, phenolics, fatty acids, terpenes/terpenoids and pyrethrin. The leaves possessed ferric-reducing power, total antioxidant activity, 2,2-diphenyl-1-picrylhydrazyl, hydrogen peroxide radical scavenging activities and membrane-stabilizing potential comparable with synthetic antioxidants such as butylated hydroxytoluene, ascorbic acid and ibuprofen. They also exhibited significant ($p < 0.05$) inhibitory property on α -amylase and α -glucosidase with IC_{50} values comparable with acarbose. For the inhibitory kinetics, NL extracts (NLE and NLA) exhibited uncompetitive and competitive inhibition on α -glucosidase and α -amylase, respectively, while TC extracts (TCA and TCE) exhibited a mixed inhibition on α -amylase. However, TCA and TCE exhibited non-competitive and mixed-mode of inhibition, respectively on α -glucosidase. TCA showed significantly ($p < 0.05$) higher *in vitro* antidiabetic activity than the other extracts and was subjected to *in vivo* toxicological and antidiabetic evaluation. In acute toxicity studies, the LD_{50} of TCA was > 5000 mg/kg b.wt with no significant ($p > 0.05$) changes in general behaviour and mortality. The sub-acute toxicological evaluation at the experimental doses revealed no significant ($p > 0.05$) alteration in the weight, biochemical, haematological and histopathological indices of the experimental animals. The induction of diabetes in high-fat diet/low dose streptozotocin-induced diabetic rats led to a loss of weight, initiation of systemic and organ oxidative stress, plasma and organ dyslipidaemia, liver and kidney dysfunction as well as observed abnormal level in other diabetes-related parameters. Upon 28-day repeated administration of TCA, these observed systemic and organ anomaly were significantly ($p < 0.05$) reversed to levels that are comparable to glibenclamide administration. *In silico* studies of 18 compounds selected from GC-MS identified phytoconstituents of the plants revealed four compounds (n-hexadecanoic acid, vitamin E, ethyl- α -d-glucopyranoside and phytol) that were potent DPP-IV, α -glucosidase and α -amylase inhibitors comparable to saxagliptin, alogliptin and acarbose. These four compounds also exhibited promising oral bioavailability, pharmacokinetics and toxicity profile. In conclusion, these plant extracts possess antidiabetic activities and do not elicit an adverse toxic effect at the doses tested. It also displays various mechanisms at which these plant extracts as well as their phytoconstituents elicit their antidiabetic action. Further studies are required to establish the antidiabetic potential and mechanism of action of ethyl- α -d-glucopyranoside and novel bioactive compounds from *N. latifolia* and *T. catappa* leaves.

Keywords: *Nauclea latifolia*, *Terminalia catappa*, Antidiabetic activity, Mechanism of action, Toxicological evaluation.